

Section 1. Biology

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SCREENING GARCINIA ZEYLANICA FOR IN-VITRO ANTIMICROBIAL ACTIVITY AND ANTI-OXIDANT ACTIVITY

Abstract. *Garcinia zeylanica* is an endemic plant of Sri Lanka which is extensively used as a culinary spice in native cuisine and used to treat wounds and gum diseases in indigenous medicine. This

study was conducted to assess the antimicrobial potency and the antioxidant activity of *G. zeylanica*. Methanolic extracts prepared from the dried fruit rind and leaves of *G. zeylanica* were tested for their antimicrobial effect against 5 bacterial species (*Escherichia coli*, *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Streptococcus pyogenes*) and a yeast (*Candida albicans*) using agar well diffusion method. The methanolic extracts exhibited significant inhibition on the growth of the tested microorganisms. The overall results of antimicrobial assay provide promising primary information for the potential of using crude extract of the dried fruit rind of *G. zeylanica* in the treatment of infections caused by bacteria and fungi. Antioxidant activity of dried fruit rind of *G. zeylanica*, the edible part used as a spice was determined using FRAP assay and ABTS assay. The extract showed interesting antioxidant activity. Owing to the elevated levels of antioxidants, *G. zeylanica* fruit can be considered as a valuable spice. Hence, *G. zeylanica* is better candidate source to discover new antimicrobial drugs.

Keywords: *Garcinia zeylanica*, antimicrobial effect, antioxidant activity.

Introduction

Infectious diseases account for one of the main challenges that modern world had to face these last few decades. Although the high proportion of effective antibiotics available, the emergence of antibiotic resistant pathogens has lowered the potency of existing antibiotics [1]. These problem lead to seek the alternative strategies to combat infectious diseases. Investigation of the efficacy of herbal extracts and formulations that have been used in folk medicinal systems could be a solution for the antibiotic resistance crisis. In Sri Lanka, many plants of Clusiaceae family are used as herbal medicines in indigenous medicine [2]. Among the plants of Clusiaceae family, plants of *Garcinia* genus, found in Africa and Asia have been reported to have antimicrobial properties [1; 3; 4]. Flavonoids and Xanthones were reported as the major phytochemicals responsible for the therapeutic potential associated with *Garcinia* species [5; 6; 7]. In Southeast Asian countries including Sri Lanka, sun-dried, smoked fruit rinds of *Garcinia* species such as *Garcinia zeylanica* (Ela goraka) and *Garcinia quaesita* (Rathu goraka) are extensively used as culinary spices [8]. *G. zeylanica* which is endemic to Sri Lanka [17] is distinguished from *G. quaesita* using its yellow rind of ripen fruit whereas the rind of *G. quaesita* is orange to red in color [2, 9]. In indigenous medicinal practices,

both *G. zeylanica* and *G. quaesita* rind extracts are used to treat gum disease, ulcers, chronic dyspepsia, asthma, diabetes mellitus and hyperlipidemia as well as to enhance digestibility [10]. Although the antibacterial activity of dried fruit rind extract of *G. zeylanica* was reported in our previous publication [3], antimicrobial potential of the crude leaf extract and the antioxidant activity of *G. zeylanica* fruit rind have not been documented to the best of our knowledge. This study therefore focuses on the antimicrobial potential of the leaf and dried fruit rind extract and the antioxidant activity of fruit rind of *G. zeylanica*. Apart from that, antioxidant capability of the *G. zeylanica* methanolic extract was analyzed considering as a valuable spice for cooking. Nowadays antioxidants have become an ongoing topic due to the anti-ageing properties of them. Most of the food categories contain medium to high level of antioxidants. These categories include berries and berry products, fruits and nuts, cereals, spices, herbs etc. Compared to animal based food products, plant based food products have generally higher antioxidant content. Among foods with plant origin, spices are well known source of antioxidants which can be included in human diet [25]. Hence, another intend was to comment on the significance of the usage of *G. zeylanica* as a spice by analyzing antioxidant capacity with appropriate protocols.

Experimental procedure

Determination of the antimicrobial activity

Preparation of the samples

Sun-dried fruit rinds (50 g) and dried leaves (50 g) of *G. zeylanica* was extracted using Soxhlet extraction apparatus separately with methanol (200 mL) as the solvent. The solvent was evaporated using rotary evaporator (40 °C, 90 rpm). Extracts were stored at 4°C until the assays carried out.

Preparation of microbial cultures

Escherichia coli (ATCC8739), *Staphylococcus aureus* (ATCC25922), Methicillin resistant *Staphylococcus aureus*, *Streptococcus pyogenes* (ATCC19615), *Pseudomonas aeruginosa* (ATCC27853) and clinically isolated *Candida albicans* were obtained from the Central Laboratory, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka. One-day old cultures of all test organisms were prepared by adjusting the turbidity to McFarland 0.5 standard using normal saline as the diluent.

Agar well diffusion method

100 µL of each prepared bacterial culture was pipetted out onto Mueller Hinton Agar (Oxoid, UK) plates separately and spread evenly using sterile cotton swabs to obtain a uniform lawn. Wells were cut on the inoculated agar plates using sterile cork borer (diameter-8 mm). Wells were loaded with dried fruit rind extract and leaf extract accordingly (50 µL each). Amoxicillin (10 mg/mL, 50 µL) was used as the positive control for MRSA, *S. aureus*, *E. coli* and *S. pyogenes* and Ciprofloxacin (10 mg/mL, 50 µL) was used so for *P. aeruginosa* while sterile distilled water (50 µL) was added as the negative control. Same procedure was carried out for *C. albicans* on Sabouraud Dextrose Agar. Fluconazole (2.5 mg/mL, 50 µL) was used as the positive control for *C. albicans*. Then the plates were incubated at 37 °C for 24 hours. After incubation, the diameter of inhibition zones measured and recorded in millimeters. The experiment was performed in five replicates.

Determination of antioxidants

Preparation of test sample

G. zeylanica sun-dried fruit rind Powder (1.0g) was taken and ethanol: water (7:3 v/v, 5ml) was added 1mL per time. Mixture was vortexed (40Hz, 1 min) each time. The contents were centrifuged and the extract was collected

Ferric reducing antioxidant power assay (FRAP)

This was conducted according to the procedure explained by Oyaizu, 1998 [18]. To prepare the FRAP reagent, acetate buffer (25.0 mL, 300 mM, pH 3.6) was mixed with freshly made Triphenyltetrazolium chloride (TPTZ) (2.5 mL, 10 mM) solution. Dilute hydrochloric acid (100mL, 40 mM) and freshly made FeCl₃ (2.5 mL, 20 mM) solutions were added to the mixture.

Prepared extract (20.0 µL) was taken and FRAP reagent (280.0 µL) was added and shake for 10 seconds. Mixture was incubated at 37 °C for 4 minutes and absorbance was measured at 593nm using Multiscan Go spectrophotometer (Thermo scientific) against a reagent blank. (20.0 µL of distilled water + +280.0 µL of FRAP reagent). The assay was performed similarly for a concentration series of 20–80 µg /mL solution of Butylated hydroxyl toluene (BHT). The reducing power was calculated according to the following formula.

Reducing power% = $[(A_1/A_0) - 1] \times 100$ (A_1 = Absorbance of reaction mixture with serum or BHT), A_0 = Absorbance of the reaction mixture with the solvent system instead of serum or BHT)

ABTS assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS[•] + cation radical was produced by the reaction between ABTS (7mM) in water and K₂S₂O₈ (2.45 mM) in 1:1 ratio. Mixture was stored in the dark place at room temperature for 12 hours. Mixture was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of the extract (2.5 µL) and ABTS solution

(247.5 μ L) absorbance was measured after 30 min of mixing at 734 nm. Percentage inhibition of absorbance at 734nm was calculated using the formula,

ABTS + Scavenging effect (%) = $((AB-AA)/AB) \times 100$, where AB is absorbance of ABTS radical

cal + methanol; AA is absorbance of ABTS radical + sample/ standard. Trolox was used as standard substance.

Results and discussion

Table 1.– Inhibition zone diameters exhibited by *G. zeylanica* leaf extract, *G. zeylanica* dried fruit rind extract and standard antibiotics

Organism	Inhibition zone diameter (mm) of leaf extract	Inhibition zone diameter (mm) of dried fruit rind extract	Inhibition zone diameter (mm) of antibiotic
MRSA	19.000 ($\pm$ 1.000)	28.800 ($\pm$ 0.837)	11.200 ($\pm$ 0.447)
<i>P. aeruginosa</i>	15.800 ($\pm$ 0.447)	24.000 ($\pm$ 0.000)	38.800 ($\pm$ 1.095)
MSSA	17.600 ($\pm$ 0.548)	29.200 ($\pm$ 1.095)	39.200 ($\pm$ 1.095)
<i>S. pyogenes</i>	18.600 ($\pm$ 0.548)	31.200 ($\pm$ 0.837)	37.400 ($\pm$ 0.548)
<i>E.coli</i> 8739	14.200 ($\pm$ 0.447)	20.800 ($\pm$ 1.095)	29.600 ($\pm$ 0.548)
<i>C. albicans</i>	0.000	18.200 ($\pm$ 0.447)	25.000 ($\pm$ 0.000)

Table 2.– Inhibition zone diameters exhibited by *G. zeylanica* leaf extract, *G. zeylanica* dried fruit rind extract and standard antibiotics, Data is expressed as

Mean $\pm$ Standard Deviation in the same column with different alphabet is significantly different ($p < 0.05$) While Mean $\pm$ Standard Deviation in the same column with the same alphabet is not significantly different. (According to the Tukey' test)

Organism	MRSA	<i>P. aeruginosa</i>	MSSA	<i>S. pyogenes</i>	<i>E.coli</i> 8739	<i>C. albicans</i>
Inhibition zone diameter (mm) of leaf extract	19.000($\pm$ 1.000) ^b	15.800(0.447) ^c	17.600(0.548) ^c	18.600(0.548) ^c	14.200(0.447) ^c	0.000(0.000) ^c
Inhibition zone diameter (mm) of dried fruit rind extract	28.800(0.837) ^a	24.000(0.000) ^b	29.200(1.095) ^b	31.200(0.837) ^b	20.800(1.095) ^b	18.200(0.447) ^b
Inhibition zone diameter (mm) of antibiotic	11.200(0.447) ^c	38.800(1.095) ^a	39.200(1.095) ^a	37.400(0.548) ^a	29.600(0.548) ^a	25.000(0.000) ^a

Table 1 describes the antibacterial activities of the *G. zeylanica* leaf extract, *G. zeylanica* dried fruit rind extract and standard antibiotics against selected pathogenic bacteria species. According to that significant antibacterial capabilities were shown by antibiotic against all selected species except the MRSA. Dried fruit rind extract of *G. zeylanica* shows consid-

erable activity against the MRSA. Statistical evaluations depict that there is a significant different among inhibition zone's diameters of *G. zeylanica* leaf extract, *G. zeylanica* dried fruit rind extract and standard antibiotic against selected bacteria species (Table 2).

Methanolic extracts of both *G. zeylanica* dried fruit rind and leaves showed significant inhibitory

effects on the growth of Methicillin Resistant *Staphylococcus aureus* (MRSA) compared to the standard antibiotic (Table 1). All test organisms except *C. albicans* were susceptible to leaf extract whereas all tested microorganisms were inhibited by *G. zeylanica* dried fruit rind extract. Further investigation of

responsible metabolite groups present in the plant extracts could eventually explain the inhibition potential of the methanolic extract of the *G. zeylanica* towards the test organisms. The reported antimicrobial activity could also be used to explain the use of this plant in folk remedies for ulcers and wounds.



Figure 1. The inhibition exhibited by *G. zeylanica* dried fruit rind extract



Figure 2. The inhibition exhibited by *G. zeylanica* leaf extract

Number of studies have reported the antimicrobial efficacy of the crude extracts of genus *Garcinia* as well their respective antimicrobial components. Mackeen et al, [11] stated the antibacterial and antifungal effects of crude methanolic extracts of

different parts of *Garcinia atroviridis*. Considerable antifungal effect of *G. atroviridis* against *Cladosporium herbarum* was notably observed with the fruit and the leaf extracts [11]. The respective antimicrobial components of this genus were found to be

xanthenes such as cowaxanthenes present in *Garcinia cowa* [12], parvifolixanthenes isolated from *Garcinia parvifolia* [13], dulcisxanthenes obtained from *Garcinia dulcis* [14], tetraprenylated xanthenes, known as scortechinones present in *Garcinia scortechinii* [15] and phloroglucinols present in *G. parvifolia* [13]. In addition, α -mangostin, found in the stem bark of *Garcinia mangostana* was effective against methicillin resistant *Staphylococcus aureus* and vancomycin resistant enterococci [16]. The antimicrobial potency of crude methanolic extracts of *G. zeylanica* corroborate with their findings.

Table 3. – Results obtained for the antioxidant assays of *G. zeylanica* fruit rind (mmol/g)

FRAP assay	ABTS
201.0 $\pm$ 0.03%	198.2 $\pm$ 0.45%

Antioxidant activity is characterized as a compound's ability to inhibit oxidative degradation, for example lipid peroxidation. The Ferric reducing power assay (FRAP) is a good indicator of potential antioxidant activity. It is based on electron transfer process rather than hydrogen atom transfer. The reducing properties are generally associated with the presence of different reductants [19]. The antioxidant action of reductant is mainly dependent on the breaking of the free radical chain by donating a hydrogen atom [20]. According to the FRAP assay *G. zeylanica* extract showed antioxidant capacity of 201.0 ($\pm$ 0.03)% while Butylated hydroxyl toluene (BHT) was used as the standard. When considering the ferric reducing capacity of the rind of the *G. zeylanica* fruit, it depicts to have a considerable capacity compared to other plant extracts [21]. Reducing capability and using the properties of electron donors to neutralize free radicals through the creation of stable materials. The effect of the reduction is to stop the radical chain reactions, which otherwise could be very damaging [22].

ABTS assay is technically simple and has been widely used for screening and routine determina-

tions. ABTS^{•+} is soluble in both water and organic solvents, which enables the antioxidant capacity of both hydrophilic and lipophilic compounds to be determined with the same basic methodology. Radical scavenging capacities were determined using ABTS. According to the ABTS assay *G. zeylanica* dried fruit rind showed 198.2($\pm$ 0.45)% antioxidant where Trolox was used as the standard substance. If an antioxidant is applied to the radicals, the presence of antioxidants induces a degree of depolarization, which reverses the formation of radical [23]. Free radicals are produced by the cells in the human body as waste substances and if they are unable to mitigate from the body, oxidative stress will be generated and reactive oxygen species can harm to the cells and body function augmenting the cancer cell production risk.

According to the results, *G. zeylanica* possess a significant amount of antioxidant content. The obtained antioxidant capacity values can be compared with well-known antioxidant rich medicinal herbal remedies. For instance, Triphala, Amalaki (*Phyllanthus emblica*) and Arjuna (*Terminalia arjuna*) used in Ayurveda and Goshuyu-tou (*Tetradium ruticarpum*), used as a medicinal herb in traditional Kampo medicine in Japan are well known antioxidant rich medicinal plants which have antioxidant values of 132.6 to 706.3 mmol/100 g [24], whereas *G. zeylanica* contains 201.0 mmol/g which can be considered as a significant antioxidant. Hence, *G. zeylanica* can be recognized as a culinary spice which contains elevated levels of antioxidants.

Conclusions

Antimicrobial potential of *G. zeylanica* against antibiotic resistant bacteria can be considered as a promising perspective of new antimicrobial drug discovery using plant sources. Apart from that, dried rind of fruit of *G. zeylanica* contains exalted amount of antioxidants proving as a healthful spice. Further, it should be focused on the bioactive phytochemical constituents of *G. zeylanica* fruit for medicinal applications.

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